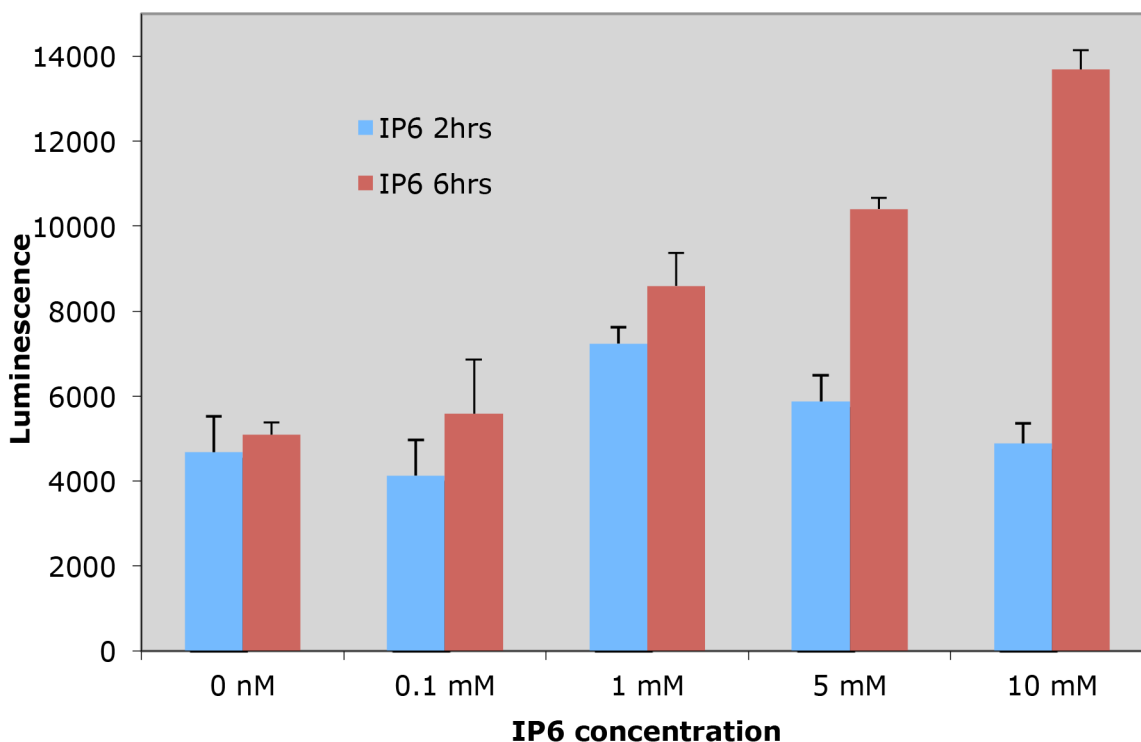


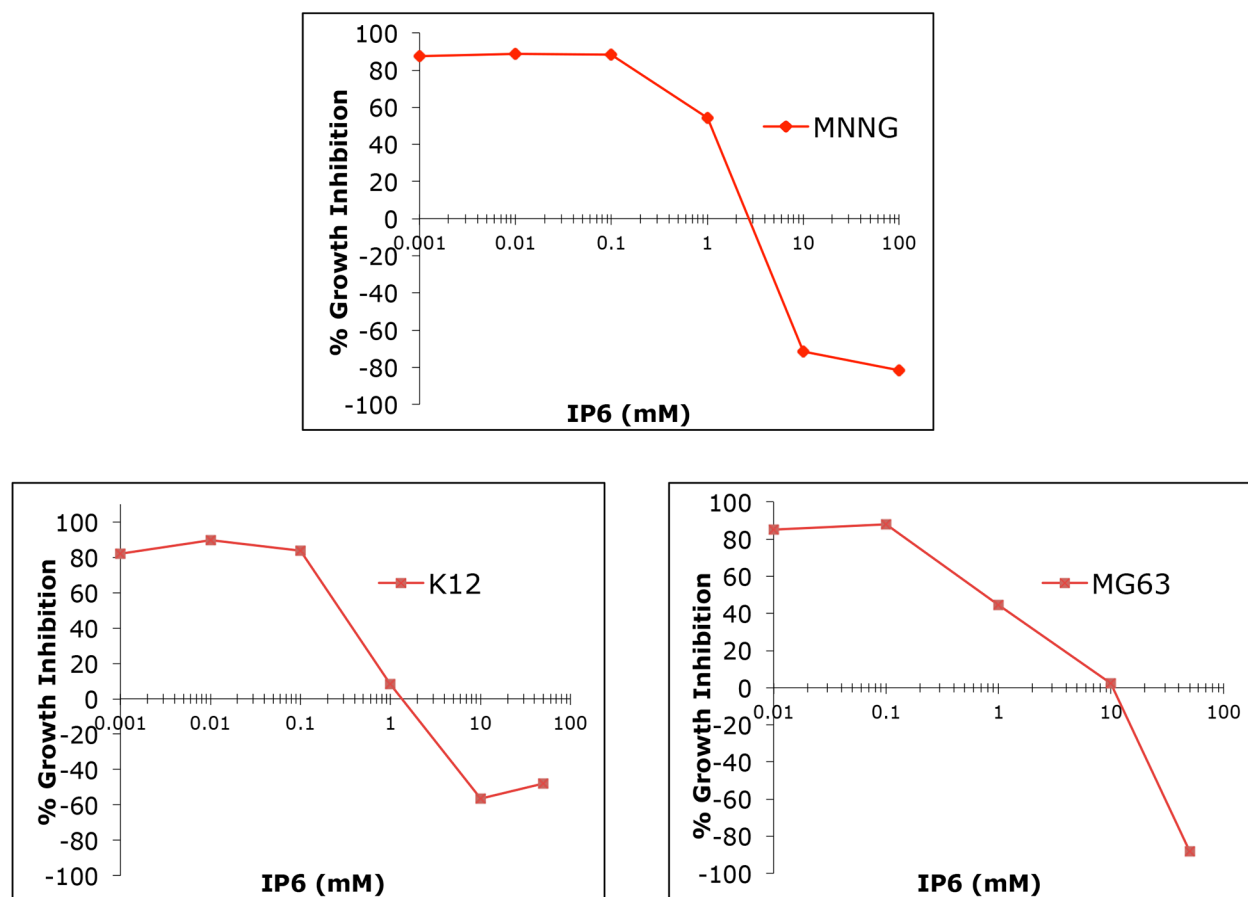
Metabolomics uncovers a link between inositol metabolism and osteosarcoma metastasis

SUPPLEMENTARY FIGURES AND TABLE

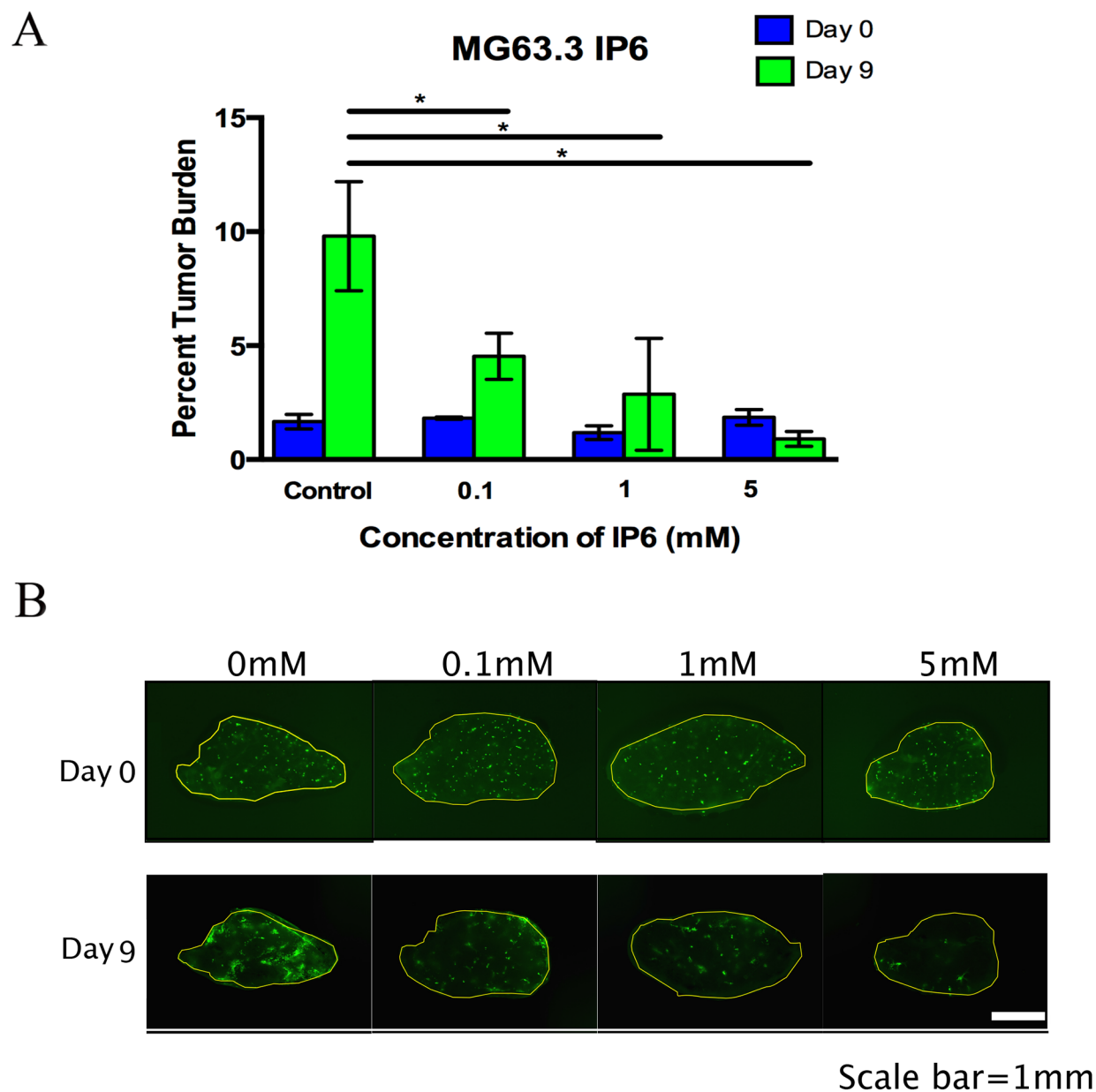
caspase 3/7 assay (MG63.3 treated with IP6 for 2hrs & 6hrs)



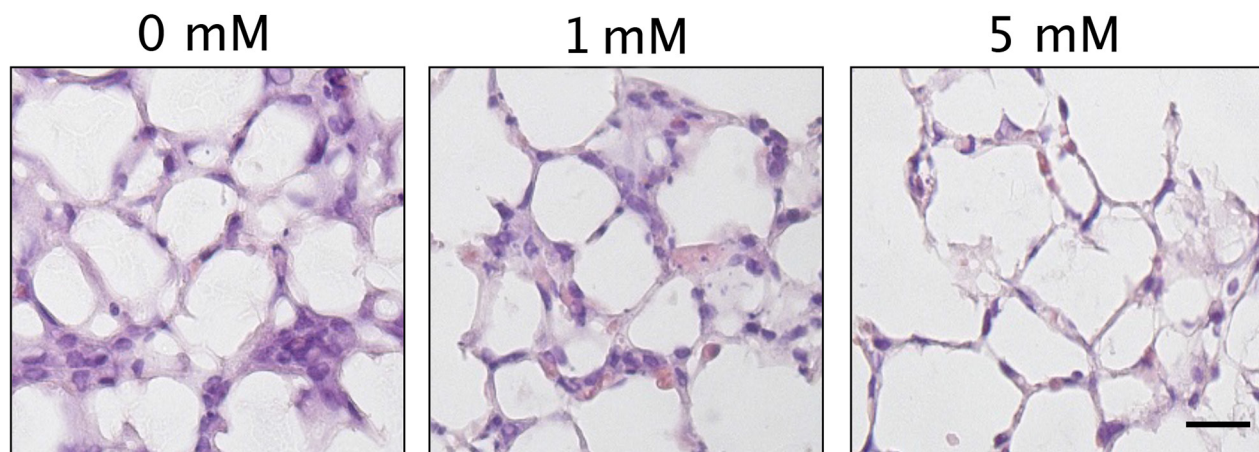
Supplementary Figure 1: Cellular caspase 3/7 activities of MG63.3 cells were increased with the addition of IP6.



Supplementary Figure 2: Effect of disrupting the inositol pathway with IP6 in highly metastatic OS cell line (MNNG) and low metastatic cell lines (K12 and MG63) *in vitro*. Cells were co-cultured for 48hrs with the indicated concentrations of IP6 followed by staining with sulforhodamine B. Growth is represented as percentage of vehicle treated control.

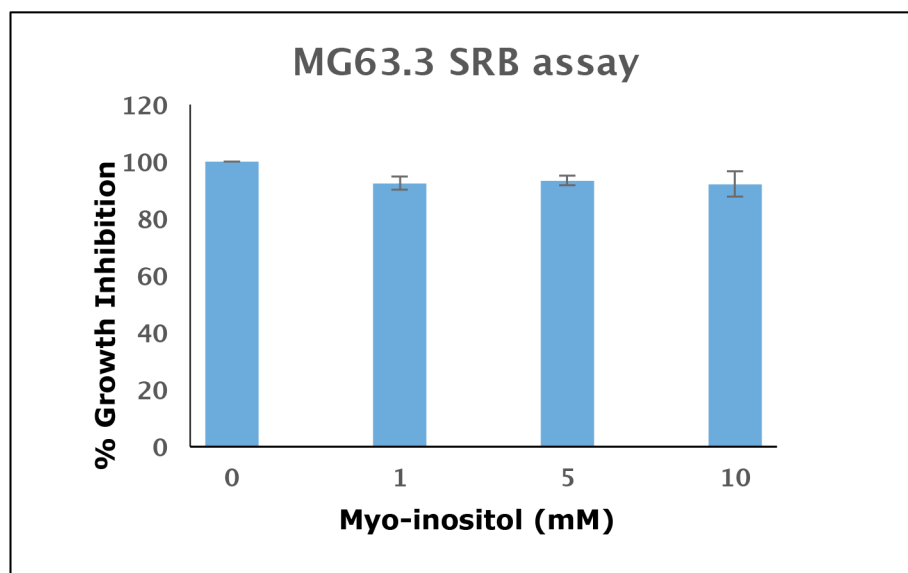


Supplementary Figure 3: Dysregulating the inositol pathway with IP6 in highly metastatic cells inhibits their ability to colonize in lung tissue in the PuMA model (A) The effects of IP6 treatment on MG63.3 cells' ability to colonize lung tissue in the PuMA model (* $p \leq 0.05$). (B) The representative images of cultured lung slides with tumor cells at day 0 and day 9.

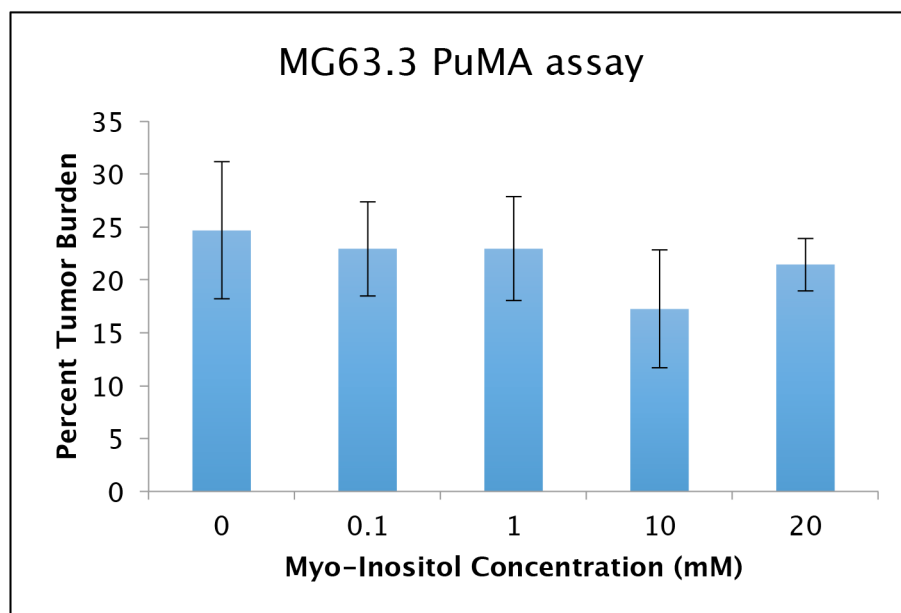


Supplementary Figure 4: Demonstration of a viable and structurally intact pulmonary architecture of IP6 treated lung. PuMA slides treated with 0 mM, 1 mM and 5 mM IP6 were fixed in neutral buffered 10% formalin (Fisher, Newark, DE, USA) for 24 hours, and then transferred to 80% ethanol. All tissues were embedded in paraffin, sectioned at 5 mm thickness, mounted on glass slides and H&E stained. Scale bar = 50 μ m.

A



B



Supplementary Figure 5: *Myo*-inositol was also tested in MG63.3 cells in an *in vitro* cell proliferation assay (A) and *ex vivo* PuMA assay (B). The inhibition of cell growth or lung metastasis was not observed at up to 10-20 mM treatment.

Supplementary Table 1: Summary of primary tumor growth, spontaneous and experimental metastasis behaviors of 4 pairs of OS cell lines

Osteosarcoma cell lines	Species	% Tumor take in mice	Days of primary tumor reach 1.5-2 cm³	% Generation of spontaneous metastasis	Median survival days of spontaneous metastasis	% Generation of experimental metastasis	Median survival days of experimental metastasis
HOS	Human	90%	Not achieved at day 200	0%	Not achieved at day 200	0%	Not achieved at day 120
HOS-MNNG	Human	100%	32	100%	65	100%	29
MG63	Human	20%	170	0%	Not achieved at day 200	0%	Not achieved at day 120
MG63.3	Human	100%	40	90%	83	100%	25
Hu09	Human	100%	180	100%	Not achieved at day 200	90%	Not achieved at day 120
Hu09-H3	Human	100%	67	100%	133	100%	60
K12	Mouse	40%	170	0%	Not achieved at day 200	20%	Not achieved at day 120
K7M2	Mouse	100%	28	80%	100	100%	29